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ORIGINAL ARTICLE

Impact of genetic variations in the MAPK signaling pathway on outcome in metastatic colorectal cancer patients treated with first-line FOLFIRI and bevacizumab: data from FIRE-3 and TRIBE trials

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Background: The MAPK-interacting kinase 1 (*MKNK1*) is localized downstream of the *RAS/RAF/ERK* and the *MAP3K1/MKK/p38* signaling pathway. Through phosphorylation *MKNK1* regulates the function of eukaryotic translation initiation factor 4E, a key player in translational control, whose expression is often upregulated in metastatic colorectal cancer patients (mCRC). Preclinical data suggest that *MKNK1* increases angiogenesis by upregulating angiogenic factors. We therefore hypothesize that variations in the *MKNK1* gene predict outcome in mCRC patients treated with first-line FOLFIRI and bevacizumab (bev).

Patients and methods: A total of 567 patients with *KRAS* wild-type mCRC in the randomized phase III FIRE-3 and TRIBE trials treated with first-line FOLFIRI/bev (discovery and validation cohorts) or FOLFIRI and cetuximab (cet) (control cohort) were included in this study. Five single-nucleotide polymorphisms in the MAPK signaling pathway were analyzed.

Results: AA genotype carriers of the *MKNK1* rs8602 single-nucleotide polymorphism treated with FOLFIRI/bev in the discovery cohort (FIRE-3) had a shorter progression-free survival (PFS) than those harboring any C (7.9 versus 10.3 months, Hazard ratio (HR) 1.73, $P = 0.038$). This association could be confirmed in the validation cohort (TRIBE) in multivariable analysis (PFS 9.0 versus 11.0 months, HR 3.04, $P = 0.029$). Furthermore, AA carriers in the validation cohort had a decreased overall response rate (25% versus 66%, $P = 0.049$). Conversely, AA genotype carriers in the control group receiving FOLFIRI/cet did not show a shorter PFS. By combining both FOLFIRI/bev cohorts the worse outcome among AA carriers became more significant (PFS 9.0 versus 10.5 months) in univariable (HR 1.74, $P = 0.015$) and multivariable analysis (HR 1.76, $P = 0.022$). Accordingly, AA carriers did also exhibit an inferior overall response rate compared with those harboring any C (36% versus 65%, $P = 0.005$).

Conclusion: *MKNK1* polymorphism rs8602 might serve as a predictive marker in *KRAS* wild-type mCRC patients treated with FOLFIRI/bev in the first-line setting. Additionally, *MKNK1* might be a promising target for drug development.

Key words: *MKNK1*, single-nucleotide polymorphisms, FOLFIRI/bevacizumab, metastatic colorectal cancer, predictive biomarker

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Introduction

Colon cancer is the fourth leading cause of cancer-related death worldwide [1]. In the last decade prognosis of metastatic colorectal cancer (mCRC) patients considerably improved mainly due to the introduction of biologicals [2]. However, to further improve prognosis and overcome treatment resistance in refractory mCRC new treatment options are eagerly awaited. Preclinical *in vitro* and *in vivo* data suggest a critical role for the MAPK-interacting kinase 1 (*MKNK1*)-mediated *eIF4E* activation in tumor development and progression [3, 4].

MKNK1 is a serine/threonine kinase localized downstream of both the *RAS/RAF/MEK/ERK* and the *MEKK/MKK/p38* signaling pathways [5]. *MKNK1* regulates diverse biologic processes including translation, cell proliferation, and differentiation through phosphorylation of different substrates such as the eukaryotic translation initiation factor 4E (*eIF4E*), heterogeneous nuclear ribonucleoprotein A1 (*hnRNPA1*), and Sprouty2 (*Spry2*) [6–8]. *MKNK1* itself is phosphorylated and activated by *p38*, *MAPK*, and *ERK*, which are localized upstream. Through phosphorylation *MKNK1* regulates the function of eukaryotic translation initiation factor 4E (*eIF4E*), a key player in translational control, whose expression is mostly upregulated in cancer patients [9–11]. In colorectal cancer patients *eIF4E* expression was markedly elevated in tumor tissue compared with adjacent normal colonic epithelial tissue [12]. Increased *eIF4E* promotes translation of mRNA encoding for proteins involved in cell cycle regulation such as c-myc, cyclin D1, apoptosis (survivin), and angiogenesis [13]. By enhancing translation of these tumor-associated RNAs, upregulated *eIF4E* stimulates tumorigenesis [14]. Noteworthily, in *MKNK1* knock-out mice normal cell development was not impaired, rendering this protein to a promising anticancer target [15]. Preclinical data also suggest that *MKNK1* stimulates angiogenesis and endothelial cell migration by upregulating angiogenic factors [16, 17], which led us to explore the impact of genetic variations in the MAPK signaling pathway, especially *MKNK1* and its substrates on outcome in patients with mCRC treated with first-line FOLFIRI and bevacizumab (bev).

Patients and methods

Study design and patient population

A total of 567 patients with *KRAS* exon 2 wild-type mCRC enrolled in the randomized phase III FIRE-3 and TRIBE trials and treated with either first-line FOLFIRI/bev (discovery and validation cohorts) or FOLFIRI and cetuximab (cet) (FIRE-3, control cohort) were included in this study. In FIRE-3 FOLFIRI/bev group bev was administered at a dose of 5 mg/kg every 2 weeks and in the FOLFIRI/cet cohort the first cet infusion was given at a dose of 400 mg/m^2, thereafter 250 mg/m^2 weekly. The FOLFIRI regimen was administered as follows: 180 mg/m^2 irinotecan, 400 mg/m^2 leucovorin, 400 mg/m^2 fluorouracil (5-FU) bolus infusion and 2400 mg/m^2 continuous infusion over 46 h. The treatment was repeated every 2 weeks until disease progression or unacceptable toxic side-effects developed [18].

The validation cohort comprised 94 *KRAS* exon 2 wild-type mCRC patients enrolled in the randomized phase III TRIBE trial and treated with the same regimen (FOLFIRI/bev) as described above. However, leucovorin was administered at a dose of 200 mg/m^2. After 12 cycles, patients in TRIBE received a maintenance therapy with 5-FU/bev until disease progression [19].

The study was approved by the local ethics committees for each participating site. All patients provided informed consent for the analysis of molecular correlates. Molecular analyses were carried out at the USC/Norris Comprehensive Cancer Center in Los Angeles. Our study was conducted adhering to the reporting recommendations for tumor marker prognostic studies [20].

Candidate polymorphisms

Potentially functional single-nucleotide polymorphisms (SNPs) within genes involved in the *MKNK1* signaling pathway were identified according to the following criteria: minor allele frequency >10% in Caucasians; potential to change gene function in a relevant matter according to public databases (https://snpinfo.niehs.nih.gov, compbio.cs.queensu.ca, https://www.ncbi.nlm.nih.gov as well as www.genecards.org) 20 June 2017, date last accessed).

Genotyping

Genomic DNA extraction was carried out from formalin-fixed paraffin-embedded tissue in the discovery and control cohorts and from blood in the validation cohort using the QIAmp DNA easy kit (Qiagen, Valencia). Six functional SNPs in six genes (*MKNK1, eIF4E, eIF4G1, 4EBP1, hnRNPA1*, and *Spry2*) were analyzed by PCR-based direct sequencing. Forward and reverse primers (supplementary Table S1, available at *Annals of Oncology* online) were used for PCR amplification. PCR fragments were then sequenced on an ABI 3100A Capillary Genetic Analyzer (Applied Biosystem) to identify the SNP. The investigator (MDB) reading the sequence was blinded to the clinical outcome data.

Statistical analysis

The aim of this study was to identify SNPs within the MAPK signaling pathway and their associations with clinical outcome in mCRC patients enrolled in two phase III randomized trials, namely the FIRE-3 and TRIBE trials. The discovery cohort consisted of patients receiving first-line FOLFIRI/bev within the FIRE-3 trial, whereas patients treated with the same regimen in TRIBE served as a validation set. The control cohort consisted of patients receiving first-line FOLFIRI/cet in FIRE-3.

Primary endpoint was progression-free survival (PFS) and secondary endpoints were overall survival (OS) and overall tumor response rate (ORR). PFS was defined as time from randomization until disease progression, death or until last follow-up in patients who were alive and remained free of disease progression. OS was defined as time from randomization until death. Patients still alive were censored at the last date of follow-up. ORR represented the percentage of patients who achieved either a complete (CR) or a partial (PR) remission according to the Response Evaluation Criteria in Solid Tumors (RECIST). Allelic distribution of genetic variants was tested for deviation from Hardy–Weinberg equilibrium (HWE) using the χ^2 test. Differences between baseline characteristics among the three cohorts were compared by using the χ^2 test. To evaluate the effects of different SNPs on PFS and OS log-rank test was used in the univariable analysis and Wald test in the Cox proportional hazards model adjusting for patient characteristics that remained significantly associated with clinical outcome in the multivariable analysis ($P < 0.10$). The adjusting factors for multivariate analyses in each cohort are described in Table 1 and supplementary Table S3, available at *Annals of Oncology* online. The associations between SNPs and tumor response were evaluated using the Fisher's exact test and a multivariable logistic regression model adjusting for ECOG performance status, previous adjuvant chemotherapy, and *BRAF* status. Significant SNPs associated with clinical outcome in the discovery cohort (FIRE-3) were tested in the validation set (TRIBE) and in the control cohort (FIRE-3).

With 247 patients (205 PFS events) in the FIRE-3 FOLFIRI/bev arm with specimen available for genotyping, we would have 80% power to

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Table 1. Association between *MKNK1* rs8602 with clinical outcomes among *KRAS* exon 2 wild-type patients

		Tumor response				Progression-free survival				Overall survival			
	N	PR + CR	SD + PD	*P* value*	*P* value**	Median, months (95% CI)	HR (95% CI)†	*P* value*	HR (95% CI)‡	*P* value*	Median, months (95% CI)	HR (95% CI)†	*P* value*	HR (95% CI)‡	*P* value*
Discovery cohort				0.17	0.12			0.10		0.15			0.12		0.095
C/C	124	73 (65%)	40 (35%)			10.8 (9.1, 13.0)	1 (Reference)		1 (Reference)		26.1 (21.5, 30.3)	1 (Reference)		1 (Reference)	
C/A	93	55 (65%)	30 (35%)			10.2 (9.2, 11.5)	1.07 (0.80, 1.44)		1.06 (0.78, 1.44)		24.7 (21.5, 28.4)	1.12 (0.80, 1.56)		1.22 (0.86, 1.73)	
A/A	19	7 (41%)	10 (59%)			7.9 (3.5, 10.1)	1.78 (1.03, 3.08)		1.81 (0.99, 3.28)		18.4 (9.8, 29.0)	1.90 (1.00, 3.60)		1.99 (1.03, 3.85)	
				0.068	**0.039**			**0.038**		0.056			0.053		0.063
Any C	217	128 (65%)	70 (35%)			10.3 (9.6, 11.7)	1 (Reference)		1 (Reference)		25.4 (23.0, 28.1)	1 (Reference)		1 (Reference)	
A/A	19	7 (41%)	10 (59%)			7.9 (3.5, 10.1)	1.73 (1.02, 2.95)		1.77 (0.99, 3.18)		18.4 (9.8, 29.0)	1.81 (0.97, 3.37)		1.84 (0.97, 3.50)	
Validation cohort				0.089	0.071			0.34		0.077			0.36		0.72
C/C	50	32 (65%)	17 (35%)			11.0 (8.4, 12.2)	1 (Reference)		1 (Reference)		33.0 (20.8, 49.1)	1 (Reference)		1 (Reference)	
C/A	36	23 (68%)	11 (32%)			11.1 (7.9, 14.3)	0.88 (0.53, 1.46)		0.84 (0.48, 1.46)		26.0 (14.4, 36.1)	1.38 (0.85, 2.25)		1.25 (0.73, 2.13)	
A/A	8	2 (25%)	6 (75%)			9.0 (2.0, 11.3)	1.75 (0.66, 4.61)		2.75 (0.97, 7.81)		36.8 (14.1, 59.5)	0.95 (0.42, 2.15)		1.16 (0.49, 2.72)	
				0.049	**0.021**			0.17		**0.029**			0.63		0.88
Any C	86	55 (66%)	28 (34%)			11.0 (9.5, 12.2)	1 (Reference)		1 (Reference)		28.8 (22.0, 36.1)	1 (Reference)		1 (Reference)	
A/A	8	2 (25%)	6 (75%)			9.0 (2.0, 11.3)	1.86 (0.72, 4.78)		3.04 (1.12, 8.27)		36.8 (14.1, 59.5)	0.83 (0.38, 1.81)		1.06 (0.47, 2.42)	
Combined cohort				**0.019**	**0.008**			0.051		0.072			0.35		0.21
C/C	174	105 (65%)	57 (35%)			10.8 (9.7, 11.9)	1 (Reference)		1 (Reference)		26.8 (22.3, 31.6)	1 (Reference)		1 (Reference)	
C/A	129	78 (66%)	41 (34%)			10.5 (9.6, 11.7)	1.02 (0.79, 1.32)		1.00 (0.76, 1.30)		25.0 (23.0, 28.4)	1.20 (0.91, 1.58)		1.25 (0.94, 1.66)	
A/A	27	9 (36%)	16 (64%)			9.0 (3.7, 10.1)	1.76 (1.09, 2.83)		1.76 (1.07, 2.88)		22.7 (14.0, 33.9)	1.26 (0.77, 2.08)		1.36 (0.82, 2.27)	
				0.005	**0.002**			**0.015**		**0.022**			0.53		0.38
Any C	303	183 (65%)	98 (35%)			10.5 (9.9, 11.6)	1 (Reference)		1 (Reference)		26.1 (23.7, 28.8)	1 (Reference)		1 (Reference)	
A/A	27	9 (36%)	16 (64%)			9.0 (3.7, 10.1)	1.74 (1.10, 2.77)		1.76 (1.09, 2.86)		22.7 (14.0, 33.9)	1.17 (0.72, 1.89)		1.25 (0.76, 2.05)	
Control cohort				0.89	0.81			0.48		0.65			0.20		0.39
C/C	136	88 (72%)	34 (28%)			9.5 (7.9, 10.8)	1 (Reference)		1 (Reference)		33.6 (27.1, 40.0)	1 (Reference)		1 (Reference)	
C/A	76	44 (71%)	18 (29%)			10.6 (9.2, 13.0)	0.89 (0.65, 1.22)		0.87 (0.62, 1.21)		25.2 (21.3, 38.3)	0.97 (0.66, 1.43)		0.84 (0.55, 1.28)	
A/A	14	8 (80%)	2 (20%)			9.7 (2.4, 13.0)	1.28 (0.71, 2.33)		1.09 (0.59, 2.03)		22.6 (13.6, 33.1)	1.81 (0.90, 3.65)		1.44 (0.68, 3.04)	
				0.73	0.53			0.33		0.66			0.076		0.26
Any C	212	132 (72%)	52 (28%)			10.2 (9.0, 10.9)	1 (Reference)		1 (Reference)		33.1 (24.4, 38.3)	1 (Reference)		1 (Reference)	
A/A	14	8 (80%)	2 (20%)			9.7 (2.4, 13.0)	1.34 (0.74, 2.41)		1.15 (0.62, 2.11)		22.6 (13.6, 33.1)	1.83 (0.92, 3.63)		1.52 (0.73, 3.17)	

*P-value is based on Fisher's exact test for tumor response, log-rank test for PFS and OS in the univariable analysis (†), and Wald test in the multivariable Cox proportional hazards regression model (‡) adjusting for age, ECOG performance status, primary tumor site, liver limited metastasis, primary tumor resection, adjuvant chemotherapy, and *BRAF* status in the discovery, control and combined cohorts; adjusting for sex, age, ECOG performance status, primary tumor site, liver limited metastasis, primary tumor resection, adjuvant chemotherapy, and *BRAF* status in the validation cohort.

**P-value was based on multivariable logistic regression model adjusting for ECOG performance status, adjuvant chemotherapy, and *BRAF* status.

PR, partial remission; CR, complete remission; SD, stable disease; PD, progressive disease. Significant *P* values are in bold.

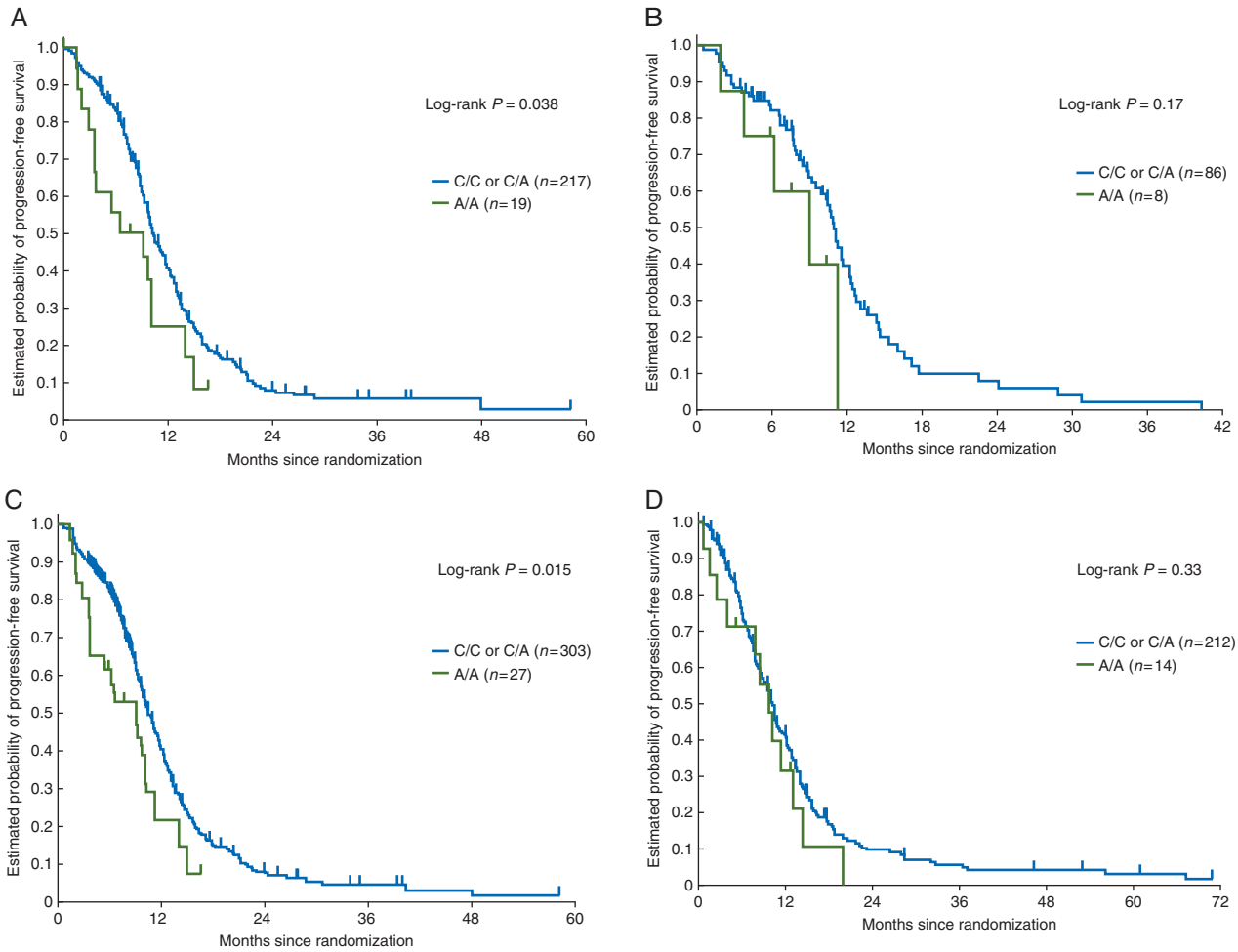

Figure 1. (A) Discovery cohort: *MKNK1* rs8602 and PFS in *KRAS* exon 2 wild-type patients (FIRE-3 FOLFIRI/bevacizumab arm). (B) Validation cohort: *MKNK1* rs8602 and PFS in *KRAS* exon 2 wild-type patients (TRIBE FOLFIRI/bevacizumab arm). (C) Combined cohorts: *MKNK1* rs8602 and PFS in *KRAS* exon 2 wild-type patients (combined FOLFIRI/bevacizumab arms of FIRE-3 and TRIBE). (D) Control cohort: *MKNK1* rs8602 and PFS in *KRAS* exon 2 wild-type patients (FIRE-3 FOLFIRI/cetuximab arm).

detect a minimum hazard ratio (HR) of 1.48–1.66 for a SNP with minor allele frequency of 0.1–0.5 on PFS using a two-sided 0.05 level log-rank test. The HR of 1.51–1.70 would be detected in the FIRE-3 FOLFIRI/cet arm with 226 patients (189 PFS events) and 1.97–2.36 in the FOLFIRI/bev arm of TRIBE with 94 patients (69 PFS events) for the same SNP using the same test and assuming the same power and allele frequencies.

All *P*-values were from two-sided Wald tests at a 0.05 significance level. All tests were carried out by using the SAS statistical package version 9.4 (SAS Institute, Cary).

Results

Baseline characteristics of the three cohorts (discovery, validation, and control sets) are depicted in supplementary Table S2, available at *Annals of Oncology* online. Shortly, the study comprised 567 *KRAS* exon 2 wild-type mCRC patients in total, 247 in the discovery cohort (FIRE-3 FOLFIRI/bev arm), 226 in the control cohort (FIRE-3 FOLFIRI/cet arm), and 94 in the validation cohort (TRIBE FOLFIRI/bev arm). The association of all 5 SNPs with outcome in the discovery cohort is outlined in supplementary Table S3, available at *Annals of Oncology* online. The allelic distribution of all genetic variants examined were within the HWE in each cohort ($P > 0.05$), except for *eIF4G1* rs2178403 SNP ($P = 0.03$). We therefore excluded this SNP from further analyses. The *MKNK1* rs8602 SNP showed significant association with PFS in patients treated with first-line FOLFIRI/bev in FIRE-3 (discovery cohort). Here, AA genotype carriers had a markedly shorter median PFS than those harboring any C allele (7.9 versus 10.3 months) in univariate analysis [HR 1.73, 95% confidence interval (CI) 1.02–2.95, $P = 0.038$] (Table 1 and Figure 1A). The same trend in PFS could be shown in patients receiving FOLFIRI/bev in the validation cohort (TRIBE). Again, patients with the AA genotype showed a decreased PFS compared with those having any C allele (9.0 versus 11.0 months, HR 1.86, 95% CI 0.72–4.78, $P = 0.17$ in univariate analysis) (Figure 1B). However, in multivariable analysis this association became statistically significant (HR 3.04, 95% CI 1.12–8.27, $P = 0.029$). Consistent with the previous findings, AA carriers in both the discovery and validation cohorts exhibited a lower ORR compared with those having any C allele (41% versus 65%, $P = 0.068$ and 25% versus 66%, $P = 0.049$, respectively). Here again, these associations became more significant in multivariate analyses in both cohorts

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($P = 0.039$ in the discovery and $P = 0.021$ in the validation sets) (Table 1). Conversely, in mCRC patients treated with first-line FOLFIRI/cet no difference in PFS could be observed between AA genotype carriers and those harboring any C allele (9.7 versus 10.2 months) in univariable (HR 1.34, 95% CI 0.74–2.41, $P = 0.33$) and multivariable analysis (HR 1.15, 95% CI 0.62–2.11, $P = 0.66$) (Table 1 and Figure 1D). Given the small number of mCRC patients carrying the AA genotype and treated with first-line FOLFIRI/bev we also conducted an analysis of the combined datasets (FIRE-3 and TRIBE) (Table 1 and Figure 1C). As expected, the association of the *MKNK1* rs8602 SNP and outcome became more significant. In the combined analysis AA carriers still show a shorter PFS than patients having any C allele (9.0 versus 10.5 months) in both univariable (HR 1.74, 95% CI 1.10–2.77, $P = 0.015$) and multivariable analysis (HR 1.76, 95% CI 1.09–2.86, $P = 0.022$). Similarly, mCRC patients with an AA genotype displayed a markedly impaired ORR compared with those harboring any C allele (36% versus 65%, $P = 0.005$ in univariate and $P = 0.002$ in multivariate analyses).

Discussion

To the best of our knowledge, we provide the first evidence that variations in the *MKNK1* gene may predict outcome in mCRC patients treated with first-line FOLFIRI/bev. While we could demonstrate that *KRAS* wild-type mCRC patients carrying any C allele have a better PFS than those with an AA genotype when treated with first-line FOLFIRI/bev in both the discovery and validation cohort, these associations were not observed in patients treated with FOLFIRI/cet.

These results suggest that the *MKNK1* polymorphism rs8602 might serve as a predictive marker in *KRAS* wild-type patients with mCRC treated with FOLFIRI/bev in the first-line setting.

MKNK1 serves as a downstream nodal point transducing signals from the *RAS/MEK/ERK* and the *MEKK/p38* signaling pathway [5]. The C allele of the *MKNK1* rs8602 variant is located in the 3'-UTR and provides a binding site for hsa-miR-1287, which regulates post-transcriptional gene expression (https://snpinfo.niehs.nih.gov (20 June 2017, date last accessed)). Through phosphorylation *MKNK1* stimulates the expression of the eukaryotic translation initiation factor 4E (*eIF4E*), which controls translation of malignancy associated mRNAs [5]. Overstimulation of *eIF4E* facilitates neoplastic transformation [21] and cancer progression via increased translation of multiple oncogenic drivers such as c-myc, cyclin D1, and survivin [4]. Niu et al. demonstrated a worse survival in colon cancer patients with high *eIF4E* tumor expression compared with those exhibiting low expression levels [22]. Due to the lack of information on chemotherapy the prognostic/predictive significance of *eIF4E* still remains to be elucidated. However, we did not observe an association between the potentially functional *eIF4E* rs36061550 variant located in the 5'-UTR and clinical outcome in mCRC patients treated with FOLFIRI/bev within the FIRE-3 trial.

Furthermore, the *MKNK1/eIF4E* axis plays a role in regulating angiogenesis through enhanced translation of vascular endothelial growth factor (*VEGF*) and fibroblast growth factor 2 (*FGF2*) [23]. In patients with bladder cancer the expression of *eIF4E* correlated with VEGF protein/VEGF mRNA ratios underlining the role of *eIF4E* in regulating *VEGF* [16]. Similarly, in breast cancer patients *eIF4E* overexpression in tumor samples was closely associated with increased expression of angiogenic factors such as *VEGF, IL-8, and FGF-2* and microvessel density [24–26]. Another study by Xu et al. demonstrated that colon cancer patients exhibiting higher *eIF4E* protein levels have an enhanced risk to develop liver metastases suggesting that overexpression of *eIF4E* results in increased angiogenesis and thereby facilitates tumor cell spreading [27]. Similarly, Nathan et al. could show that an increase in *eIF4E* during head and neck tumorigenesis correlates with higher *VEGF, b-FGF* and microvessel density [28].

The influence of the *MKNK1-eIF4E* axis on angiogenesis may explain why we observe differences in outcome only in patients treated with FOLFIRI/bev but not in those receiving FOLFIRI/cet. One might assume that *MKNK1* activation might circumvent VEGF blockade of bevacizumab by selectively activating alternative angiogenic factors to further promote angiogenesis.

Therefore, targeting *MKNK1* may be a promising approach to enlarge our treatment armamentarium against mCRC and to overcome resistance in patients treated with bevacizumab based chemotherapy. Available data on *MKNK1* are almost limited to experimental models indicating its role in tumorigenesis [3, 15, 29] and preclinical studies showing activity of *MKNK1* inhibitors against different types of solid tumors [9, 14]. Due to these promising results, a clinical phase I–II trial evaluating the safety and activity of daily oral *MKNK* inhibitor (eFT508) has been initiated and is currently recruiting patients with treatment refractory advanced solid tumors (NCT02605083, https://clinicaltrials.gov/ct2/show/NCT02605083 (20 June 2017, date last accessed)).

In our study, the significant association of the *MKNK1* SNP rs8602 with PFS in *KRAS* exon 2 wild-type mCRC patients treated with first-line FOLFIRI/bev in two independent phase III studies confirms our initial hypothesis and support furthermore previous findings from other groups suggesting an influence of *MKNK1* in upregulating angiogenesis [23–26].

Due to the limited number of patients with *KRAS* exon 2 mutations enrolled before the protocol amendment in FIRE-3 (discovery cohort), and the low frequency of *MKNK1* rs8602 AA genotype carriers, we did not analyze the association of this SNP on outcomes in *KRAS* exon 2 mutant patients, as it would not allow us to draw any firm conclusion. Similarly, sample size restrictions and the low prevalence of AA carriers precluded us from performing further subgroup analyses in *RAS* wild-type/mutant or *BRAF* mutant patients. Therefore, we intentionally restricted our analysis to *KRAS* exon 2 wild-type patients, who represented the intention-to-treat population in FIRE-3 (discovery cohort).

The strength of our study is that we included 567 *KRAS* wild-type mCRC patients enrolled in three different cohorts of two phase III randomized trials who were treated with either FOLFIRI/bev or FOLFIRI/cet. Secondly, our findings obtained in the discovery cohort could be confirmed in the validation cohort.

A limitation of our study is the small number of patients harboring any AA genotype. However, by combining both cohorts of FIRE-3 and TRIBE FOLFIRI/bev arms the decreased PFS among AA carriers became more evident.

In conclusion, our results suggest that the *MKNK1* polymorphism rs8602 might serve as a predictive marker in *KRAS* wild-type patients with mCRC treated with FOLFIRI/bev in the first-line setting.

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Disclosure

The authors have declared no conflicts of interest.

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